EXPRESSION OF GLYCOSYLATED HUMAN INFLUENZA HEMAGGLUTININ PROTEINS

BACKGROUND OF THE INVENTION

The present invention relates generally to the production of human influenza hemagglutinin proteins in baker's yeast, *Saccharomyces Cerevisiae* employing recombinant DNA technology. More particularly, the present invention relates to the production in yeast of glycosylated human influenza hemagglutinin proteins which mimic native influenza hemagglutinin proteins present in humans and animals to thereby provide a potentially effective vaccination agent.

The publications and other reference materials referred to herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference. For convenience, the reference materials are numerically referenced and grouped in the appended bibliography.

The influenza virus is a well known human and animal pathogen which causes pandemics and major epidemics in humans and animals. Although the disease is usually relatively mild in healthy individuals, it can be quite serious in elderly individuals and/or those who have chronic physical ailments. In addition to pain and suffering, the financial losses from lost work time due to influenza epidemics are quite substantial. Accordingly, the prevention of outbreaks of influenza is of great economic and social value.

Influenza is caused by a virus vector which invades and infects host organism cells, disrupting their useful functions. Vaccines prepared from killed or attenuated influenza virus have been in use since the early 1940's. These conventional vaccines are usually prepared from chick embryos in which the virus is grown. Live virus is subsequently killed before it is used as a vaccine. Since the whole virus is used in vaccination, numerous problems have resulted from the use of such vaccines, including adverse side reactions, toxic effects and other problems inherent in the production of a vaccine from the killed or attenuated virus. Accordingly, there has been a great deal of interest in developing an effective vaccine against influenza which does not require the use of whole virus either attenuated or killed.

The influenza virus is a segmented negative strand enveloped RNA virus which codes for at least 10 proteins. Three of the proteins are the membrane proteins: hemagglutinin (HA), neuraminidase (NA), and matrix protein (M) (**1**). These membrans proteins are assembled into virus particles during maturation of virus as it "buds" through the host-cell membrane. Of the three membrane proteins, HA and NA are integral membrane glycoproteins.

Recently, a great deal of interest has been generated in studying both HA and NA. Their primary as well as 3 dimensional structure have been determined (**57,58**). However, HA is quantitatively the major surface glycoprotein of influenza virus (**2**) and the antigen against which neutralizing antibodies are elicited (**3, 4**).

HA of the A/Hong Kong/1968 virus is a trimer of 224,640 molecular weight (MW). It may be solubilized from the viral membrane by bromelain digestion, which removes a 5,406 MW C-terminal hydrophobic (anchoring) peptide from each subunit. This observation, extended by the results of subsequent primary sequencing experiments places the hemagglutinin in a class of integral membrane proteins characterized by a three-domain structure with a large hydrophilic, carbohydrate-containing domain on the external surface of the membrane, a small, uncharged hydrophobic peptide of 24–28 amino acids spanning the membrance, and a small, hydrophilic domain (10–55 amino acids) on the internal side of the membrane (**57**).

The HA chain is typical of membrane glycoproteins and is initially synthesized as an immature polypeptide including an N-terminal hydrophobic signal peptide. The signal is subsequently removed as part of the process by which the polypeptide is transported across and anchored into the membrane as the mature polypeptide. Each polypeptide chain of the mature trimer is glycosylated at seven sites with a total carbohydrate of 13,000 MW (19% by weight) (**57**). The glycosylation sites are found in the HA where there is an amino acid triplet beginning with asparagine and ending with either threonine or serine. The sites are located at amino acids Nos. 11–13, 56–58, 125–127, 268–270, 480–482 and 539–541. In higher animals, the sugars are first attached in rough endoplasmic reticulum and further processed, and new sugar molecules are attached in Golgi complex. These sugars are attached at the glycosylation sites and the native hemagglutinin contains fucose, galactose, high mannose, glucose, sialic acid and other complex sugars.

The advent of recombinant DNA techniques has aided greatly in our understanding of the structural features that determine the biological and antigenic properties of the HA of influenza virus (**5, 6, 7**). As a result of the development of recombinant DNA techniques, polypeptides corresponding to the mature HA protein have been expressed in *Escherichia coli* (**8,9, 10**). The microbial production of HA in *E. coli* has generated a great deal of interest in utilizing the recombinant HA as subunit vaccines against influenza (**11**).

Laver et at., :The Antigenic Sites on Influenza Virus Hemagglutinin. Studies on Their Structure and Variation," *Structure and Variation Influenza Virus, Supra.*, p. 295, and Wiley et at., Nature, 289, 373 (1981), report on the amino acid sequences of importance with respect to antigen determinants. Specifically, based upon their work, it can be predicted, in general, that amino acides 30 to 275 of the mature hemagglutinin protein contain at least one and probably the most important of the several antigenic determinants. Specifically, they show that amino acides 140 to 146 of A/Memphis/102/72 (H3) represent a site important for an antigenic determinant. This corresponds in structure to amino acids 153 to 159 A/WSN/33 (H0). In accordance with the present invention, it is possible to express each of the human influenza hemagglutinin polypeptides, or proteins thereof which contain at least one antigenic determinant site, located as defined above.

Co-pending U.S. patent application No. 239,301 was filed on Mar. 2, 1981 and is assigned to the same assignee as the present application.

In this application, various plasmid vectors are disclosed which code for polypeptides corresponding to antigenic portions of mature HA (i.e., HA without the signal peptide). The plasmids are used to transform *E. coli* to express the hemagglutin polypeptides which were demonstrated to be useful as vaccination agents. The mature HA expressed by *E. coli* is not glycosylated due to *E. coli's* inherent inability to produce glycoproteins.

Since one of the goals of a vaccine is to mimic the natural state of the viral antigen as closely as possible, it